THE BACTERIAL ORIGIN OF THE GUMS OF THE ARABIN GROUP.

X.—The Pararabin Gum of Sterculia.

(*Bact. pararabinum*, n.sp.)

By R. Greig Smith, D.Sc., Macleay Bacteriologist to the Society.

The gum which sometimes exudes from specimens of Sterculia has been investigated by Maiden,* who found that it consisted essentially of arabin and pararabin.† The latter is presumably a modification of the former, and differs from it in being insoluble in water. Pararabin also differs from arabin, as well as from metarabin or cerasin, in not being hydrolysed upon boiling with dilute sulphuric acid.

I have already shown that arabin is the product of *Bact. acaciæ*, and that metarabin is produced by *Bact. metarabinum*. It would, therefore, be interesting if an organism capable of forming pararabin could be isolated. Such a result would not only show how diverse can be the gum-products of bacteria, but also how the gums, which were supposed to be secretions of the higher plants in a pathological condition and to have been produced from cellulose, are really the byproducts of the bacterial fermentation of sugars.

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* Maiden, Pharm. Jour. [3] xx., 1890, 381.

† Pararabin found in beet-root, carrots, agar-agar, is amorphous, swells in water, is soluble in dilute mineral acids, and is precipitated therefrom by alkalies or alcohol; upon warming with alkalies gives arabin, with dilute H_2SO_4 no sugar, does not decompose carbonates. — Dammer und Rung, "Chemisches Handwörterbuch."

Specimens of the fruit, etc., of *Sterculia diversifolia*, showing numerous gum-drops upon the seed-capsules and twigs, were sent to me by Mr. H. W. Potts, Principal of the Hawkesbury Agricultural College. The substance of the capsules was saturated with a mucilage which oozed through insect punctures in the pods, and formed gum-drops upon the outside as it dried. From these specimens I hoped to obtain an organism capable of forming pararabin.

Bacteria were readily obtained, in the manner that I have previously described, from portions of the punctured fruits, from the very young entire fruits (measuring about 1 cm. in length) and from unpunctured twigs.

The colonies were those of *Bact. acaciæ*, and of races of another bacterium which was closely investigated. Since the bacteria were obtained from the twigs and unpunctured young pods, it is clear that the plant had not been infected by the same insects that made the holes through which the gum exuded. Infection must have occurred at another place, possibly on the stem, and at a less recent date.

When infected upon the surfaces of plates of saccharose-potato-agar, the unknown bacterium grew as a whitish slime which could be readily removed. A watery suspension of the slime was coagulated by copper sulphate (1% and 10%), ferric chloride, aluminium hydrate, lead acetate (10%), basic lead acetate, baryta water, milk of lime, and silver nitrate (5%). Upon standing a sediment separated out from the slime, and the almost clear supernatant liquid also gave precipitates with the reagents enumerated.

When the specimens of fruit arrived at the laboratory, several pods were soaked in water, and the mucilage which exuded was precipitated with alcohol. But a small precipitate was obtained from a fairly mucilaginous solution, and when this small quantity was dissolved or diffused in water it was precipitated by lead acetate, baryta water, copper sulphate, silver nitrate, and slightly with ferric chloride. These reactions were sufficient to show that the Sterculia mucilage and the bacterial slime have certain

common properties, and that the organism which I had separated would eventually be found to be a pararabin-producer.

The coagulation of the slime by all the reagents enumerated is not characteristic of Sterculia slime, for I have already shown that cane gum is also precipitated or coagulated. The slime of *Bact. persicæ*, the arabinan-galactan organism of the peach, etc., when in strong solution, is also coagulated by these reagents. The slimes of *Bact. persicæ* and *Bact. vascularum* differ from the Sterculia bacterium slime, in that they are not resolved by treatment in the autoclave at three atmospheres' pressure into a deposit of bacterial remains and an almost clear or turbid supernatant gummy fluid. In this respect there was an agreement between the Sterculia bacterium slime and the slimes produced by the arabin and the metarabin bacteria.

The turbid solution of the gum, when treated with alcohol, gave a precipitate which consisted of large curdy masses and flocculles. As the saline matter was removed during the process of eliminating the last traces of sugar, the alcohol threw down a precipitate, and at the same time produced a "milk." The precipitate was only partly soluble in water; the alcohol had gradually converted much of the carbohydrate into an insoluble modification. Saline flocculating agents, such as potassium chloride or better barium chloride, coagulated the "milk," and by dissolving the precipitate in water an opalescent solution was obtained.

The insoluble gum dissolved readily in dilute hydrochloric acid, but boiling 1% sodium hydrate simply coagulated the diffused or swollen carbohydrate, leaving a clear solution. The solubility in dilute acid and insolubility in dilute alkali are characteristic of pararabin.

The slime was obtained by growing the bacterium upon the surface of an agar medium containing 5% saccharose and 50% potato juice. The potato juice and the medium should not be neutralised at any time during its preparation. The natural acidity undoubtedly favours the production of slime, causing it to be more gummy and less opaque; evidently there are less bacterial cells and more gum. When neutralised potato juice is

used there is obtained a smaller quantity of a thick white slime. Whether the increase of gum is due to the acid reaction of the medium or to the partial inversion of the saccharose is not clear; but, since reducing sugars are present in potato extract, it is probable that the natural acidity is the essential factor in stimulating the bacteria to slime-production rather than to reproduction.

The races of the organism.—Upon saccharose-potato-agar the bacteria always produced slime—that is to say, if the bacteria grew at all, slime was produced. Three races of the bacterium had been isolated, and these differed chiefly in the temperatures between which they grew. Race i., produced as much slime at 18° as at 24°; at 30° and 37° the slime was less. Race ii., grew equally well at 18°, 24°, 30° and 37°. Race iii., grew equally well at 18°, 24° and 30°, but did not grow at 37°. Race i., produced the largest quantity of slime, and it is this race which was used in the work connected with the action of the organism.

The slimes (*i.e.*, carbohydrate together with the bacterial cells and other products) which were produced by these races behaved differently to certain chemical reagents. For example, the slime of race i., was coagulated by copper sulphate, neutral lead acetate and barium hydrate, while races ii. and iii. were not. The slimes of all the races were coagulated by ferric chloride, aluminium hydrate, basic lead acetate, and milk of lime. The coagulation of the slime by many reagents is therefore not distinctive.*

When the gum was separated from the bacterial cells and other products and while in the soluble condition it behaved somewhat differently with these reagents. Curdy precipitates were obtained with alcohol, barium hydrate, basic lead acetate and ferric chloride. Neutral lead acetate and copper sulphate gave no precipitate. Copper sulphate followed by sodium hydrate gave a light blue precipitate which contracted but did not darken upon

* The slime of *Bact. persicæ* differed in its behaviour to copper sulphate according to the temperature of incubation of the cultures. These Proceedings, 1903, p. 339.

heating. In this respect it is similar to the arabin and metarabin gums. Fehling's solution sometimes did and sometimes did not precipitate the gum. These tests were made with the gum of race i., after the slime had been heated in the autoclave and the separated gum had been repeatedly precipitated with alcohol to remove the sugars.

The bacterium also produces slime in fluid culture. A medium containing saccharose 50, peptone 2, ammonium chloride 1, potassium phosphate 1, magnesium sulphate 0·5, chalk 10, and water 1000 grms. was, after sterilisation, infected and incubated at the air temperature (25°). By the 10th day, the solution had become very viscous, and from it a small quantity of slime was obtained by treatment with alcohol. When made into an emulsion with water, the slime behaved to reagents like that grown upon the surface of agar.

The products of hydrolysis.—The slime from agar was purified by repeated precipitation with alcohol from aqueous emulsion until it was found to be free from sugars. The gum was then obtained from the slime and its hydrolysis was attempted by boiling with 5 % sulphuric acid. At the end of six hours a portion was abstracted, neutralised and tested for reducing sugars. Fehling's solution gave a pale blue flocculent precipitate, but there was no reduction. At the end of twelve hours Fehling's solution gave the same negative reaction. The carbohydrate had not been hydrolysed, and in this respect it is similar to pararabin, which is not hydrolysed upon boiling with dilute sulphuric acid.

The sulphuric acid solution was divided into two and one of the halves was evaporated to half volume (= 10 % sulphuric acid) and boiled for six hours. The other half was nearly neutralised with baryta water, filtered and evaporated down with 50 c.c. of normal phosphoric acid until the solution darkened in colour and evolved the odour of burning sugar. The solution was then diluted to 33 c.c. (= 5 % solution) and boiled for six hours. From the solution which had been boiled with 10 % sulphuric acid, a few milligrams of an osazone which melted at 177-180° were obtained. The small quantity of osazone from the solution,

which had been treated with phosphoric acid, melted at 168-169°. Both osazones were put together and dissolved in weak alcohol. The alcohol was boiled off and a water-insoluble, lemon-yellow, crystalline powder which melted at 191° was obtained. From the hot water solution crystals separated out on cooling; these dried on porcelain as a brownish-yellow skin which melted at 170°. The appearances and melting points of these osazones indicated galactosazone, and a mixture of arabinosazone and galactosazone.

As the quantities of sugars obtained by the above methods had been too small to enable the osazones to be separated in a practically pure state, a further quantity of gum was hydrolysed. This test differed from the former in the gum having been obtained in fluid media containing saccharose. The possibility of agar contaminating the gum was thus prevented. The carbohydrate was freed from saccharose and reducing sugars by repeated precipitation with alcohol from aqueous solution or suspension. The curdy gum finally obtained was moistened with 2 c.c. of strong sulphuric acid and was then rubbed into a paste in a glass mortar. When the mixture had become brownish in colour, 25 c.c. of water were added, and, after transferring to a flask, the mixture was boiled for 9 hours under a reflux condenser. The solution, which contained reducing sugars, was neutralised with barium carbonate, filtered, evaporated, clarified with aluminium hydrate and finally treated with phenylhydrazine mixture* and heated on the water-bath for two hours. The solution was cooled and the residue, after filtration, was dried on porcelain and then treated with ether to extract the tarry impurity. The osazones melted at 175-177°.

The undoubted mixture of osazones was successively treated with (1) hot water, (2) hot dilute alcohol, and (3) hot strong alcohol. The first fraction consisted of a mass of yellow crystalline needles which dried on porcelain as a brown skin and melted at 162-164°. Further treatment with hot water extracted arabin-

* Phenylhydrazine 1 c.c., glacial acetic acid 1 c.c., water 0·5 c.c.

osazone melting at 159°. The second fraction dried as a loose yellow powder with a brown tinge. It melted at 184-186°. The third fraction dried as a loose yellow powder which melted at 190-191°. This was dissolved in hot alcohol, and hot water was added until a workable precipitate settled out. The clear yellow powder so obtained was galactosazone melting at 194°.

The slime has thus been seen to contain a carbohydrate which had the properties of pararabin, viz., upon drying it became insoluble, and this modification was insoluble in dilute alkali, soluble in dilute acid; it could not be hydrolysed by boiling with dilute acid, but by appropriate treatment with strong sulphuric acid it was hydrolysed to arabinose and galactose.

Invertase is not secreted.—Many bacteria while producing gum from saccharose invert a part of the sugar to levulose and dextrose, one of which may be utilised. This organism does not secrete invertase. The supernatant liquid from saccharose-chalk cultures did not reduce Fehling's solution. Instead of reducing the fluid, the gum formed a precipitate which coagulated on boiling.

The influence of various sugars, &c., upon slime-formation.—In the culture media hitherto employed saccharose had been the carbohydrate nutrient. But as other carbohydrates might be capable of replacing saccharose, experiments were made to investigate this question. The results showed that dextrose, levulose, galactose, mannite and glycerine could replace saccharose. Of these levulose and glycerine were better than the others, and better even than saccharose. The following carbohydrates were useless: raffinose, lactose, maltose, inulin, starch and dextrin. The experiments were made with a peptone and chalk fluid, and also with nutrient meat-agar, to both of which media the carbohydrates had been added previous to sterilisation. The fluid cultures corroborated the results obtained with the agar medium. Potato-extract-agar was also used, but as this medium contains reducing sugars, it did not show clearly the effect of the added carbohydrates. There was one exception, however. The addition of glycerine produced a gelatinous growth, the bacteria being

apparently contained in comparatively large masses of slime. These masses were also noted when glycerine had been added to the nutrient agar. They lay loosely upon the agar and could be scraped together into a gelatinous heap.'

Since the gum can be formed from glycerine, this substance should be much better than saccharose when the gum is required in quantity, for the residual glycerine could be more easily removed. Furthermore, a whiter gum could be obtained; the saccharose solutions during sterilisation, etc., become brownish in colour, and as this colour is conveyed to the purified gum, its solutions are not colourless.

The other byproducts of the fermentation of saccharose.—A saccharose-peptone-medium contained in a small flask was infected with the organism and connected with another flask containing baryta water. The air inlet was sealed with a screw-clip and the air outlet was connected with a tube of soda-lime. No aerial carbon dioxide could therefore gain access to the apparatus. At the end of five days the air from the culture flask was drawn through the baryta water, when a copious formation of barium carbonate occurred. Carbon dioxide is thus a byproduct in the fermentation of saccharose.

The supernatant liquid from a 20 days' culture containing chalk and saccharose was treated with barium hydroxide and boiled under an inverted condenser in order to saponify alcoholic esters. The liquid, after cooling, was filtered and distilled in a partial vacuum until about one-third had passed over. The residual fluid was evaporated down and reserved for the extraction of the acids. The distillate was distilled and the process repeated until about 10 c.c. of fluid had been obtained. As this contained ammonia it was made acid to litmus with phosphoric acid and distilled at atmospheric pressure. The first 2 c.c. of distillate were absorbed with anhydrous sodium carbonate and distilled. The first drops that passed over were collected and the boiling point determined by Siwoloboff's method. The fluid boiled at 78° and burned with a blue flame. It also gave the iodoform reaction, and undoubtedly was ethyl alcohol.

The residual fluid reserved for the extraction of acids was evaporated to small bulk, acidified with sulphuric acid and filtered. The residual chalk, with adhering salts and liquid from the culture flask, was also treated with dilute sulphuric acid until all the chalk had been decomposed and the suspension was strongly acid; it was then filtered. The two filtrates were reserved for extraction with ether.

The two residues of sulphate of barium and calcium were dried in the air, then ground to a rough powder in a mortar, and finally extracted with ether. After the evaporation of the ether, the extracted acids were treated with hot water, when an oily acid separated out. This was washed with water, dried, dissolved in ether and filtered. After the ether had evaporated, the fatty acid, which was solid at the ordinary temperature, was melted and sucked into capillary tubes in which the acid crystallised in clusters of silky needles. These melted at 42·5°, and apparently consisted of lauric acid.

The reserved filtrates were extracted with ether in Schoorl's apparatus, and, after the ether had been distilled off, the residual solution of the acids was added to the liquid from which the lauric acid had been obtained. The volatile acids were driven off in a current of steam. The proportion of these to the residual or non-volatile acids was as 1 : 9·7, or roughly as 1 : 10. The volatile acids consisted chiefly of butyric, with small quantities of acetic and formic acids. The partial separation of the acetic and butyric acids was effected by the treatment of the calcium salts with strong alcohol as recommended by Schoorl,* and the recognition was made by the odour of the acids and the ethyl esters. The solution of the non-volatile acids was evaporated and allowed to crystallise overnight, when prisms of succinic acid separated out. These sublimed, gave a buff precipitate with ferric chloride and ammonia, and melted at 180°. The method of Schoorl was then followed, when a further separation of succinate was effected. No other acids were obtained.

* Schoorl, Jour. Soc. Chem. Ind., xix., 567.

The acids produced during the growth of the organism in saccharose solutions are therefore succinic, lauric, butyric, acetic and formic, the relative proportions being in that order. Besides these acids, ethyl alcohol and carbon dioxide are formed.

The organism did not produce characteristic growths upon the various media. The most distinctive characters were perhaps the production of a gummy slime on saccharose-potato-agar, and of a pronounced viscosity in fluid media containing certain sugars, etc., and chalk. As pararabin has never before been shown to result from bacterial activity, it is probable that the organism is new,* and I have accordingly named it *Bacterium pararabinum*, n.sp. (*Bacillus pararabinus*, n.sp., by Migula's nomenclature).

BACTERIUM PARARABINUM, n.sp.

Shape, etc.—The organism appears as an actively motile, short thick rod with rounded ends. It tends to form long rods, chains, and threads in old cultures. The young cells, as taken from a 24 hours' agar culture, measure 0·6-0·7 : 0·8-1μ. The flagella may be single and terminal, or numerous and peritrichous; up to seven have been observed upon one cell. The rods colour readily with the ordinary stains, and are decolorised by the Gram method. Spores were not observed.

Temperature, etc.—The growth temperatures have been noted on page 544. The bacterium is aërobic; no growth occurred under the mica plate.

Nutrient agar plate.—At 30° the colonies are circular, raised translucent-white and gummy. Microscopically they are rounded and finely granular, with irregular curved structures scattered here and there. The deep colonies are oval, rounded or lenticular, and coarsely granular.

Glucose-gelatine plate.—In two days at 22° the colonies were white, rounded, raised and gum-like, although they did not draw

* *Bact. gelatinosum betæ*, Fritz Glaser, a dextran bacterium, appears to be the most closely related slime-forming organism.

into threads when touched with the needle. Microscopically they were coarsely granular and clouded, with curved or coiled structures scattered throughout the colony. The deep colonies were round and dark, with short delicate cilia radiating from the margin.

Nutrient agar stroke.—The growth appears translucent-white, raised, moist or fat glistening, smooth or rough; the margin remains straight or becomes lobular. The consistency is either thin or gelatinous.

Saccharose-potato-agar stroke.—The growth may be (1) raised, luxuriant, translucent-white and non-gravitating; (2) white, gummy and gravitating; or (3) thin, white, spreading, with gas production in the condensed water.

Nutrient gelatine stab.—The growth along the needle track appears filiform, with a white, raised or depressed, glistening or dull nail-head. As the nail-head spreads outwards, the centre sinks, and a tubular or crateriform pit is formed, below which the medium is locally liquefied.

Glucose-gelatine stab.—The stab becomes filiform, with a dry, glistening white nail-head, either raised at the margin and depressed in the centre, or flat and spreading. The nail-head eventually becomes crateriform from the consumption of the medium, which is liquefied below the centre of the film. The medium may or may not darken.

Potato.—The growth is yellowish-white, thin, glistening and scattered; it becomes raised, and buff-white and appears gummy or fatty.

Bouillon.—The medium becomes very turbid with a loose flocculent sediment and slight surface ring. The indol reaction was obtained, and in nitrate-bouillon the nitrate was reduced to nitrite.

Milk.—The medium is not affected.

Summary.—The gum of *Sterculia diversifolia* consists of a mixture of arabin and pararabin. The arabin is produced by

Bact. acaciæ. Another organism—*Bact. pararabinum*, n.sp.—was isolated from the gummed fruits, etc. Upon solid media and in solutions containing saccharose, dextrose, levulose, galactose, mannite or glycerine, a slime is formed. By appropriate treatment this yields a soluble pararabin gum which upon dehydration becomes insoluble, and this modification is soluble in dilute acid and insoluble in dilute alkali. It is not hydrolysed by dilute acid, but strong acid converts it into arabinose and galactose. The bacterium does not secrete invertase, and in solutions of saccharose it forms gum, ethyl alcohol, carbon dioxide, succinic, lauric, acetic, butyric and formic acids.

Errata.—On p. 119 of these Proceedings, in third line from bottom, *for* 67·08 *read* 64·68; and on p. 348, in line 5, *for* gelatine *read* galactan.